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Research Note

Rapid extraction of genomic DNA from small plant leaf samples

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Introduction: The isolation of genomic DNA is essential for many molecular biology applications. The presence of polyphenolic compounds and polysaccharides in plants such as grapevine (Steenkamp *et al*. 1994) makes the isolation of DNA and the polymerase chain reaction problematic (Bryant 1997). Nevertheless, several successful DNA extraction methods have been developed based on standard protocols (Doyle and Doyle 1990, Harding and Roubelakis-Angelakis 1994, Lodhi *et al.* 1994, Steenkamp *et al*. 1994, Labra *et al*. 2001) and commercial kits are also routinely used with grapevine (Qiagen Dneasy Plant mini-kit, Qiagen, Hilden, Germany).

Toxic or difficult to handle compounds such as ß-mercaptoethanol, phenol/chloroform and liquid nitrogen, make these procedures difficult for processing when numerous samples must be analysed with a small amount of tissue. Furthermore, these techniques are time consuming and can be expensive.

We developed a simple and rapid protocol that can be used to isolate PCR- DNA quality from a 5 mm leaf disk from grapevine and various other plant species. This protocol overcomes the difficulties with few steps and simple handling.

Material and Methods: Plant material: At different developmental stages leaves were harvested from greenhouse-grown and field-grown *Vitis vinifera*, *Nicotiana tabacum* and field-grown *Lycopersicum esculentum*, *Rosa sp.*, *Malus domestica*, *Pirus communis*, *Prunus domestica* and *Salix sepulcralis*. We used embryogenic callus from *Vitis vinifera*, as well.

DNA extraction: DNA was extracted from a 5 mm leaf disk, cut with a standard paper punch. The leaf disk was rapidly and mechanically disrupted with a piston pellet in an Eppendorf tube containing 100 µl extraction buffer (1x Phosphate buffered saline (13.7 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4, pH 7.3), 2 % Sarkosyl, 2.5 % Polyvinyl-pyrrolidone-40, 0.25 % SDS). The sample was incubated at 95 °C for 10 min, and centrifuged at 10,000 g for 1 min at 16 °C. After transfering 70 µl of supernatant to a clean Eppendorf tube, DNA was precipated with 70 µl isopropanol for 5 min at room temperature. The mix was centrifuged 5 min at 11,000 g at 16 °C and the pellet dried 1 min in a vacuum speed and resuspended in 50 µl Tris HCl 10 mM, pH 8.5.

Polymerase chain reaction: Amplification reactions were performed in a total volume of 50 µl with 4 µl of resuspended DNA, and primers for the *18S rDNA* region: 0.35 µM forward primer 5'-AACGGCTAC-CACATCCAA-GG-3', 0.35 µM reverse primer 5'-TCAT-TACTCCGATCCC-GAAG-3', 200 µM dNTP (Invitrogen), 1.5 mM $MgCl_2$, 1x PCR Buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl; Invitrogen) and 0.2 unit Platinum® Taq DNA Polymerase (Invitrogen). The PCR was carried out in a GeneAmp® PCR System 2700 thermocycler (Applied Biosystems). The cycling program was: 2 min at 94 °C followed by 35 cycles of 40 s at 92 °C, 1 min at 57 °C and 1 min at 72 °C and a final extension step of 7 min at 72 °C. The different PCR products were separated by electrophoresis on a 1.2 % agarose gel.

Results and Discussion: To assess the PCR-DNA quality we amplified a *18S rDNA* gene region. This sequence is highly conserved among a wide range of plants. The expected 378 bp fragment was amplified successfully from all samples (Fig. 1) and reproducibly due to at least three separate experiments. *Vitis vinifera* DNA purified using our method was also used for microsatellite analysis with optimal results (Fig. 2).

Amplification of the expected DNA fragment from grapevine leaves was obtained even if the mechanical disruption was omitted (Fig. 3), the leaf disk being only

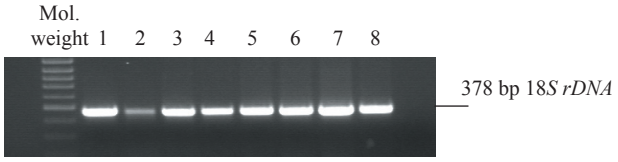

Fig. 1: PCR amplification of extracted genomic DNA from leaves of *Vitis vinifera* (**1**), *Nicotiana tabacum* (**2**), *Lycopersicum esculentum* (**3**), *Rosa sp.* (**4**), *Malus domestica* (**5**), *Pirus communis* (**6**), *Prunus domestica* (**7**), *Salix sepulcralis* (**8**). The PCR primers used were designed to detect a 378 bp fragment of the *18S rDNA* region.

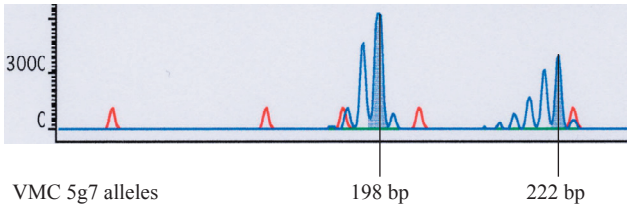

Fig. 2: Microsatellite analysis of genomic DNA from grapevine leaves after extraction using our technique. DNA was analysed using one pair of primer flanking the microsatellite region: VMC 5g7 (*Vitis* Microatellites Consortium, Dr. Rosa Arroyo) marked with the fluorophore FAM. The amplification products were separated by capillary electrophoresis and detected with an ABI PRISM 310 Genetic Analyser (Applied Biosystem), using HD400-ROX as an internal size standard.

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incubated in the extraction buffer. In this case, the intensity of the band was weaker in comparison to that obtained after mechanical disruption. No difference in the intensity of the band was observed with the DNA extracted from callus with or without disruption (Fig. 3).

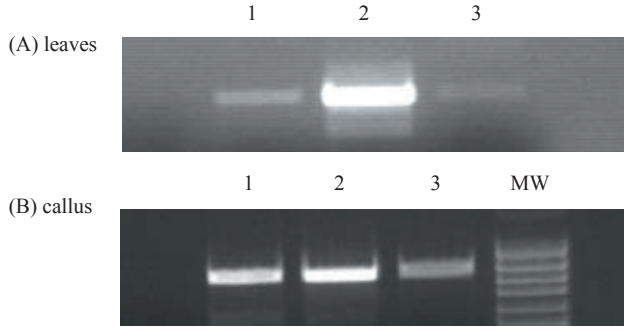

Fig. 3: PCR amplification of genomic DNA from grapevine leaves (A) or callus (B). After extraction using our technique with (2) or without (1) mechanical disruption or using SIGMA REDExtract-N-Amp™ Plant PCR Kit (3). PCR primers used were designed to detect a 378 bp fragment of the *18S rDNA* region.

We compared the PCR-DNA quality of purified DNA using our technique with that of DNA produced with the SIGMA REDExtract-N-Amp™ Plant PCR Kit. This kit allows to rapidly extract genomic DNA from a 5 mm leaf disk after incubation with an extraction solution at 95 °C for 10min and a neutralization step, without mechanical disruption. The SIGMA kit is adapted for DNA extraction from leaves and embryogenic callus of grapevine (Fig. 3). But, the technique we have developed and used with or without the mechanical disruption allowed a stronger amplification for grapevine leaves and calli as compared to the commercial technique (Fig. 3).

In comparison to the usual protocols for extraction of genomic DNA from leaves our protocol presents many decisive advantages: few handling, no plant tissue freezing in liquid nitrogen, no organic extraction, no silica-gel-membrane technology. This protocol is particularly adapted for rapidly processing numerous samples with small amounts of tissue (10 samples in 30 min).

Our protocol is adapted for DNA extraction from tissues such as grapevine leaves, for which it is very difficult to obtain high quality DNA due to the presence of significant amounts of polysaccharides and polyphenolic compounds. But it is also efficient with various other species such as tobacco, tomato, rose, apple tree, pear tree, plum tree and weeping willow.

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